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In vitro – In vivo Correlation : Dissolution Profile and Pharmacokinetic of Calcium Carbonate Tablets Marketed in Yemen

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Abstract

The study aimed to validate an indirect colorimetric based on classical approach for evaluation the in-vitro/ in-vivo correlation of calcium carbonate tablets marketed in Yemen. The colorimetric was validated and used for the determination of calcium concentration in different brands tablet and body, calcium dissolution testing in vitro (IV) and pharmacokinetic in vivo (IV) through blood samples of 18 health males volunteers. On the other hand, IV/ IV correlation was estimated in this study. The results showed that the photometric method was precise (RSD : 1.27%) and accurate (mean recovery: 100.17%). The study proved poor quality of generic products marketed in Yemen and this could be attributed to the bad pharmaceutical manufacturing and absences of efficient regulatory systems. Also, this method was instead the atomic spectrophotometer method required by USP monographs for the assay of calcium dissolution testing. The similarity factor (f2) and mechanism release in vitro of generic versus original affected by different manufacturing process of calcium products and this was clear in this study. In addition, the results proved that the bioequivalence of generic products versus original product was not available (IV/IV correlation was not available for all products). Poor quality of some products marketed in Yemen may affect the efforts to control hypocalcaemia and providing high quality of drug products will significantly reduce diseases. A non– linear IV/ IV correlations were established between various dissolution and bioavailability parameters for three commercial brands of calcium tablets using level A IV/IV correlation approach. Therefore, with proper standardization of methods of assessment, in vitro dissolution parameters can be used to predict in vivo bioavailability of these calcium tables marketed in Yemen.

Keywords: *Validation, Colorimetric, Calcium, In vitro, In vivo, Correlation .*

1. Introduction

Calcium carbonate is a dietary supplement used when the amount of calcium taken in the diet is not enough. Calcium is needed by the body for healthy bones, muscles, nervous system, and heart. This medication may also be used to control low blood calcium levels in people who do not get enough calcium from their diets or for kidney failure patients [1-3]. This product was administrated orally. For the chewable form, chew the medication well before swallowing. For the liquid form, shake the bottle well before each dose. Several studies have been done on leaching of calcium in different pharmaceutical and biological matrix were reported, European Pharmacopoeia (EP) measured the calcium carbonate as raw material based on titration potentiometric method [4]. United State Pharmacopoeia (USP) measured the calcium in pharmaceutical forms (tablet , powder and syrup) using the atomic absorbance method [5]. British Pharmacopoeia (BP) used titration method [6]. The objective of our study to validate of indirect colorimetric method for quantification of calcium carbonate in tablet form with aid International Conference Harmonization (ICH) guideline. The second objective is to assess quality of reference and generic calcium carbonate pharmaceutical products marketed in Yemen. The third objective, to compare of dissolution profile of original and generic calcium carbonate marketed in Yemen.

2. Materials and Methods

1.2. Standards, chemicals and instruments

The materials included kit of calcium assay ((potassium cyanid with ethanolamine as reagent A , methylthymol blue with hydroxyquinoline as reagent B) , tube without anticoagulant (QCA Company , Aspain) , sphygmomanometer , Balance , Meter (China Brand), syringe , Tourniquet and lancets ., Spectrophotometer of Apple , (USA) , centrifuge of Remi (India) ., Frability and Hardness of pharma-test (Germany) , Dissolution of Erweka (Germany). In addition, drug products namely calcium bicarbonate and drugs were used namely calcium carbonate namely 1500 equivalent 600 calcium mg in tablet was purchased from licensed pharmacy (Hodeidah City), Yemen.

2.2. Volunteers

The study included 18 health volunteers that were males. Their age ranged between 18 and 24 years**.**

2.3. Methods of validation

A referenced analytical methods for assay of calcium in serum was re–validated based on EDQM and USP requirements aid protocol of International Conference harmonization (ICH Q2)

which were carried out for assay and dissolution profile of calcium carbonate . The standards (calcium) were prepared in separate by accurately weighting 0.64 mg /ml as stock and diluting to obtain on (0.32 , 0.16, 0.08 and 0.04) mg / ml of calcium RS . These solutions were mixed for 5 min.

2.4. Principle of calcium assay

The level of calcium was determined by using calcium-MTB(Methylthymol Blue) methods . Calcium in the sample reacts with methylthymol blue in alkaline medium forming a coloured complex that could be measured by spectrophotometry . Hydroxyquinoline was included in the reagent to avoid magnesium interference . The absorbance was measured by using spectrophotometer Spectro 23 RS at 610 nm [7] .

2.5. Dissolution test " In vitro" :

Dissolution test is carried out to obtain information about the possible differences in the bioavailability . The author used USP monograph was used with tolerances for calcium tablets not less than 75 % (Q) of the labelled amount that are dissolved in 30 minutes and used paddle method (Apparatus 2), at a stirring rate of 75 rev/min for 45 minutes (16). The volume of dissolution medium was 900 ml namely distilled water at 37 C. The amounts of calcium dissolved in 15 minutes, 30 minutes and 45 minutes were determined by a validated method namely colorimetric method at wavelength of 546 nm in filtered portions of the solution under test in comparison with a standard solution of USP calcium and was expressed as a percentage of the label claim [6].

2.6. Pharmacokinetic of original versus generic Calcium Tablet (In vivo)

First, 18 health females volunteers should be fasting and the numbers of getting blood of volunteers are 4 from (8:00Am to 11:00 Am) .we should make centrifuge for every volunteers blood The blood of volunteers was centrifuged in every hour. After that the first test for volunteers blood was tested without any calcium tablet and after getting first volunteers blood, we should directly give them calcium tablet was given to them directly . Then, wait one hour for the The second step at for getting volunteers blood was after one hour. After that, and repeat getting blood from the volunteers was repeated at 10:00 Am and 11;00 Am .

2.7. IV/IV correlation (Level A)

Level A correlation is to define a direct relationship between in vivo data such that measurement of in vitro dissolution rate alone is sufficient to determine the biopharmaceutical rate of the dosage form.

2.8. Data Analysis

2.8.1. Validation of data analysis

The excel software 2010 was used to compute the validation results of the colorimetric analytical method as well as to obtain the linearity, precision and accuracy (expressed by recovery) according to equations 1 – 3 , respectively [8-12] . .

$$y = ax + b \qquad \textbf{Eq. (1)}$$

where b is the intercept of the straight line , a is the slope of the line , y is the response (absorbance) and x is the introduced concentration of calcium .

$$RSD\ (\%) = \frac{a}{x} x100 \qquad \textbf{Eq. (2)}$$

$$\mathrm{R}\ (\%) = \frac{\mathrm{x}^{\wedge}}{\mu^{\wedge}}.100 \qquad \textbf{Eq. (3)}$$

where, μ^ is the mean of the introduced concentrations and x^ is the estimate of the mean concentration obtained .

2.8.2. Dissolution data analysis

Also , the similarity (f2) tests were applied to the dissolution data for generic calcium products versus original product. The f_2 (50 – 100) of generic drugs (Tt) versus reference (Rt) at number of time point (n) are calculated by the following equation [13,14] :

$$f_2 = 50.log\left\{[1 + \left(\frac{1}{n}\right)\sum_{i=1}^{n} (R_t + T_t)^2]^{-5}.100\right\}$$

Eq. (4)

For knowing the mechanism of original and generic calcium release from formulations, the data were fitted to Korsmeyer's (log cumulative percentage of drug released versus log time) .

$$\frac{Mt}{M\infty} = at^n \qquad \textbf{Eq. (5)}$$

where Mt is the amount of drug released at time t, M∞ is the amount of drug released after infinite time (total drug in a dosage form), a is the Korsmeyer's dissolution rate constant and n is the release exponent . Erosion diffusion (0.45 > n) that is defined " Erosion - represents the loss of material from a matrix due to disintegration and determines the release rate of the drug" , Fickian diffusion (n = 0.45) that occurs by the usual molecular diffusion of the drug due to a chemical potential gradient" , Non - Fickian diffusion anomalous diffusion " (0.45 < n < 0.89) that is defined " release mechanism i.e., drug release is by coupling of Fickian diffusion" ,Case II relaxation release transport (n = 0.89) that is defined " Case-II transport : release is the drug transport mechanism associated with stresses which swell in dissolution medium. Super case II transport (0.89 < n) that is called " anomalous diffusion" [15] .

2.8.3. Pharmacokinetic data analysis

First-order

If the amount of drug A is decreasing at a rate that is proportional to A, the amount of drug A remaining in the body, then the rate of elimination of drug A can be described as:

$$Log\ A_B = logA_B^o - kt/2.303 \qquad \textbf{Eq. (6)}$$

where k is the first-order rate constant, A_B is the drug in the body at time t, $A°_B$ is the drug in the body at time= 0.(Dose).

One-compartment model

The relationship described in (Figure 1.2a) where can be plotted on a log Cp can be plotted vs time graph and the graph will then show a linear relation; this represents a one-compartment model.

Volume of distribution (Vd)

At zero concentration (Cp^o), the amount administered is the dose, D, so that If the drug has a large Vd that does not equate to a real volume, e.g. total plasma volume, this suggests that the drug is highly distributed in tissues. On the other hand, if the Vd is similar to the total plasma volume this will suggest that the total amount of drug is poorly distributed and is mainly in the plasma.

$$Vd = \frac{D^0}{Cp^0} \qquad \textbf{Eq. (7)}$$

Half-life

The time required to reduce the plasma concentration to one half its initial value is defined as the half-life (t1/2).(19)

$$t_{1/2} = \frac{0.693}{k} \qquad \textbf{Eq. (8)}$$

Concentration Maximum (C max)
The maximum concentration of the drug in the curve (peak).

$$C_{max} = B.\left(e^{-kt_{max}}.e^{-k_a t_{max}}\right) \qquad \textbf{Eq. (9)}$$

$$\boldsymbol{B = \frac{F \times Ka \times D^\circ}{Vd \times (ka - k)}} \qquad \textbf{Eq. (10)}$$

Note : B = cp°

Time maximum (T max)
Time at which the maximum concentration reached. the parameter cant be determined graphically because it would not be accurate therefore instead its it was determined from the following equation.

$$t_{max} = \frac{2.303 \log (k_a/k)}{k_a - k} \qquad \textbf{Eq. (11)}$$

K and Cp° from the diagram .Area under the curve ($AUC_{0-\infty}$), Mean the area under the curve infinity which give information about bioavailability (21,9)

$$AUC_{0-\infty} = \frac{C_p{}^0}{k} \qquad \textbf{Eq. (12)}$$

2.7.4. IV/IV correlation data analysis

When various dissolution parameters were correlated with various bioavailability parameters based on regression equation.

3. Results and Discussion

3.1. Validation of analytical method
3.1.1. Specificity
The specificity of the colorimetric method for quantification of calcium in tablet that was tested to obtain an indication of the possible interferences from the drug substance with other components at routine application analysis.

3.1.2. Linearity
Five calibration of calcium standard were prepared to evaluate the relationship between the absorption and the concentration (**Figure 1 and Table 1**). The results showed that the relationship linearity was evaluated in a concentration range of 0.10 mg /ml to 0.66 mg /ml, covering the normal range of concentrations obtained when analyzing the calcium with slope 1.2809 , intercept 0.0029 and correlation coefficient (R^2) equal to 0.9998.

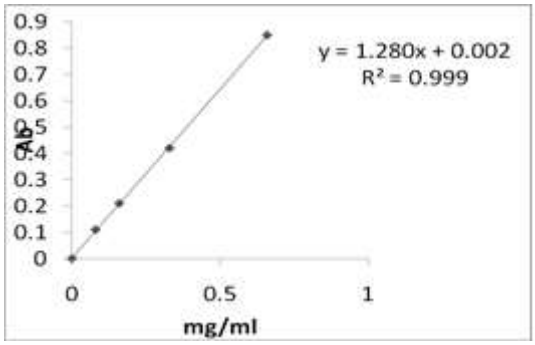

Figure 1 . Linearity of colorimetric method

3.1.3. Accuracy

The accuracy parameter was measured based on the standards at three levels concentrations ranging from approximately 0.66, to 0.16 mg/ml. Recoveries of validated method ranged from 98.84 % to 101.39 % with a mean of 100.17 % (**Table 1**). The homogeneity of the recoveries through all concentrations levels was measured and the results obtained showed no significant different between them ($p > 0.05$)

3.1.4. Precision

The repeatability of the method was tested by analyzing three levels of recoveries samples ranged from 0.66, to 0.16 of calcium ; the percent relative standard deviation (RSD %) was 1.27 % (**Table 1**).

Table 1 : Results of analytical method validation

Validation parameters	Values
Linearity	
Slope	1.28
Intercept	0.002
Correlation coefficient (R^2)	0.999
Precision (RSD %)	1.27 %
Accuracy (%)	
Mean	100.17 %
Range	98.84 % – 101.39 %

3.2. Real content of calcium in tablet

The results of dosage of calcium content in tablets were reported in (**Table 2)**. The content of active ingredient in tablets was found to be within normal range of USP in three products A, B and C namely 99.77 % , 102.80 % and 104.26 %, respectively.

Table 2 : Results of real content of calcium in tablets

Products	Values
Product A	99.77 %
Product B	102.80 %
Product C	104.26 %

3.3. Dissolution profile

The results of calcium release (%) (reference 1 and 2, and 3 as a generic marketed in Yemen, tablet 600 mg in USA dissolution condition namely HCl 0.1 N have shown in (**Table 3**). However, the reference (product A) faster than the generic (products B and C) . In brief , all products were not accepted based on USP rules (Q = ≤ 75 %) at 30 min (**Figure 2**). It's may be due to the different of excipient types as well as their physical properties (particles size) or the type of the manufacturing processes using to produce the final product. But the calcium in reference tablet released more than (≤ 75 %) at 45 minute. On the other hand, the results of *f*2 of generic calcium carbonate versus reference original in the USP dissolution condition were 14.05 and 13.88 respectively. These results were out of the normal values ($50 \leq f_2 \leq 100$) due to the bioequivalence in vitro was not available (**Table 4**). Also , the release (%) of calcium mechanism from the reference and generic tablets in the standard condition was determined based on a Korsmeyer - Peppas model , and the diffusion exponents were 1.20 , 0.441 and 0.489 , respectively (**Table 5**) . The n value of the product A that indicated to super case-II transport (0.89 < n) , The diffusional exponent of product C in standard condition indicated non-Fickian type of release mechanism (0.45 < n < 0.89). While the product B that indicated Erosion diffusion (0.45 > n) .

3.5. Bioavailability

The results obtained showed that C_{max} of the product A were higher than C max of the products B and C. In addition , the AUC∞ of the product A was higher than the AUC∞ of the product B while C AUC∞ of the product C is higher than the product B (**Table 6 and Figure 3**). the bioavailability of product A is the highest . And the bioavailability of product B is the lowest. On the other hand , the results proved that the bioequivalence of generic products (B and C) versus original (A) were not available (**Table 7**) .

3.5. In vivo-in vitro correlation (Level A)

A non linear relationship between the fractions of drug absorbed and the fractions of drug released that was obtained for all products (A,B and C) because the R^2 less than 0.995 , In brief , the R^2 of product A was better than products B and C , Since the absorption cannot "keep up“ with the dissolution (**Figure 4**).

Table 3 : Dissolution profile of calcium carbonate tablets marketed in Yemen

Descriptive Analysis	Product A			Product B			Product C		
	15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
X	15.5	64.35	75.3	1.3	2.02	11.01	3.38	4.06	7.72
RSD (%)	20.5	19.86	1.54	9.39	3.87	1.24	1.08	5.95	1.24

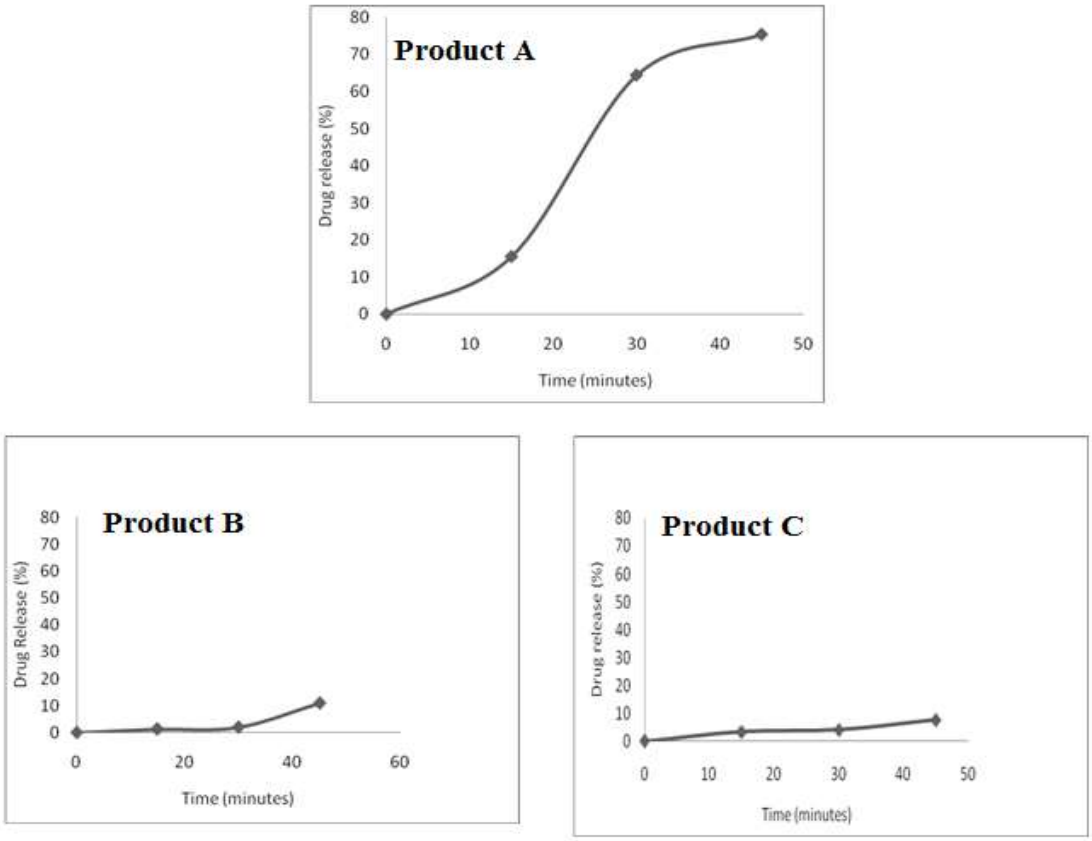

Figure 3. Dissolution profile of different brands of calcium carbonate marketed in Yemen

Table 4 : Similarity factor of generic products versus original product

Similarity Factor	Product B versus Product A	Product C versus Product A
f_2	14.05	13.88

Table 5 : Mechanism of drug release in vitro based on Korsmeyer - Peppas model

Products	Slope exponent release (n)	Type of Mechanism release in vitro
Product A	1.20	Supper Case II Transport
Product B	0.441	Erosion diffusion
Product C	0.489	Non -Fickian diffusion anomalous diffusion

Tablet 6 : Bioequivalence of generic products versus original product

Parameters	Product B versus Product A	Product C versus Product A
C max (%)	59.6	13.9
AUC $_{0-\infty}$ (%)	67.8	85.2

Tablet 7 : Pharmacokinetic of calcium carbonate parameters

Parameters	Product A	Product B	Product C
Order	First order	First order	First order
Type of compartment	One	One	One
Cp (µg/ml)	1109.380	553.671	668.671
K (hr^{-1})	00.142	00.0744	00.3134
t ½ (hr)	44.880	99.315	22.211
Vd (L)	55.485	111.535	88.737
T max (hr)	00.811	00.895	00.659
C max (µg/ml)	3373.861	2222.984	551.826
$AUC_{0-\infty}$(µg.hr/ml)	2278.666	1188.951	2237.453

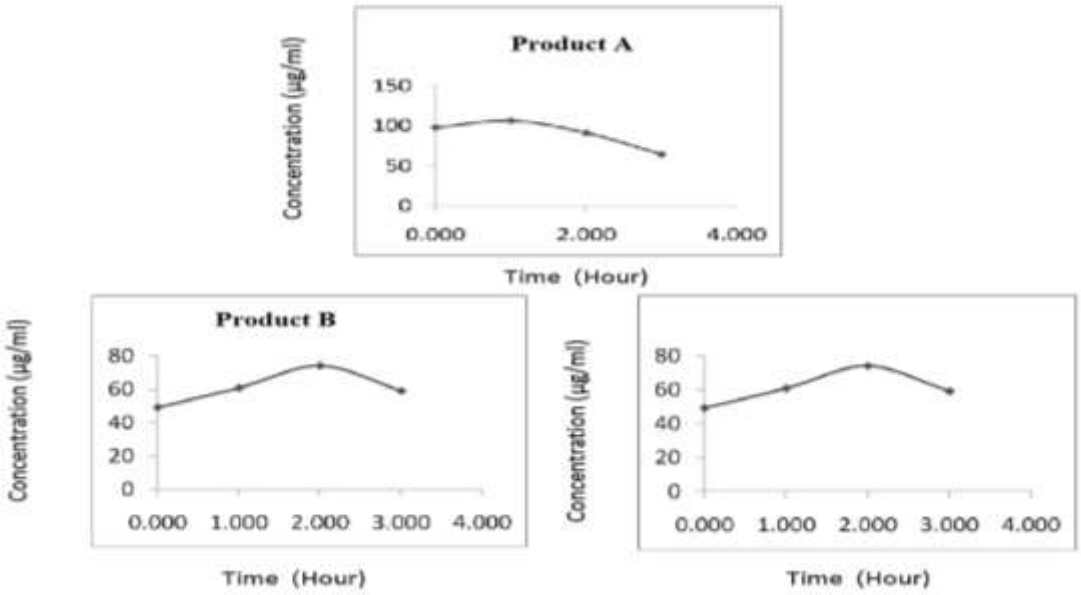

Figure 3 . Pharmacokinetic of different brands of calcium carbonate marketed in Yemen

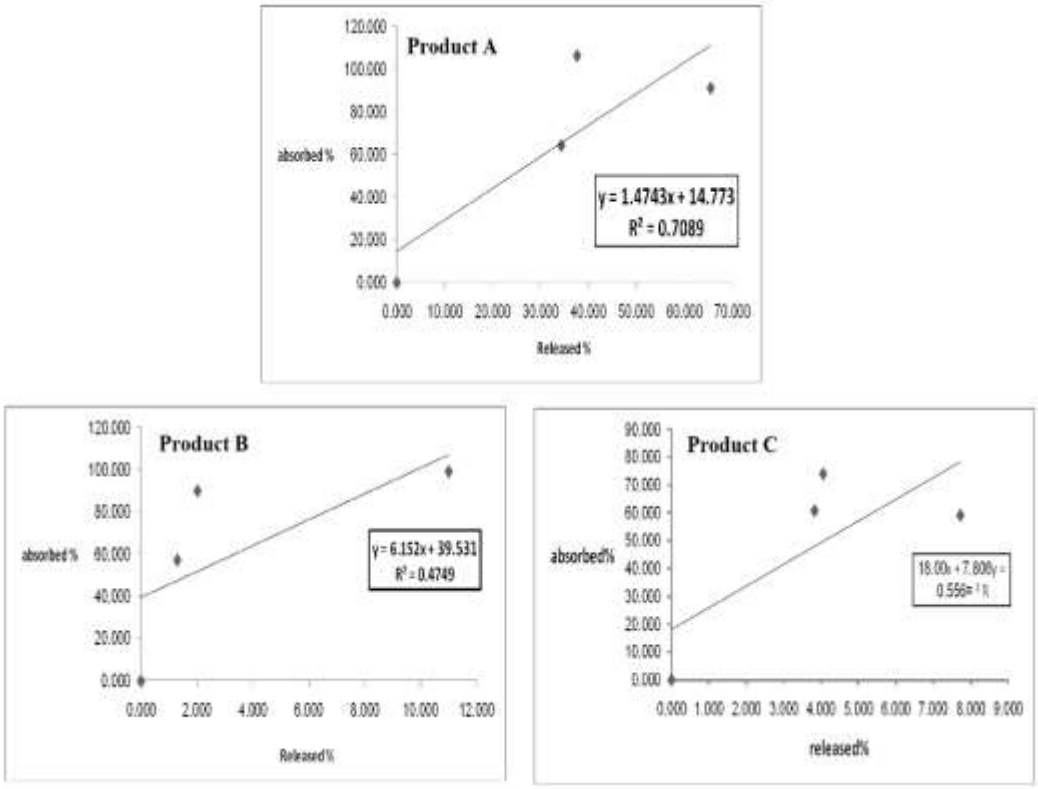

Figure 4. Level A IV/IV correlation of calcium carbonate marketed in Yemen

4. **Conclusion**

Indirect colorimetric assay was validated using a classical approach for the assay of calcium in solid pharmaceutical forms. Our finding showed poor quality of some products marketed in Yemen that due to the bad manufacturing and the lack of authority regulatory systems. Poor quality of these products may affect the efforts to control hypocalcaemia. Also, this method was instead the atomic spectrophotometer method required by the USP monograph for the assay of calcium dissolution testing. The similarity factor and mechanism release in vitro of generic products versus original product affected by different manufacturing process of calcium products and this was clear in this study . In addition , the results proved that the bioequivalence of generic products versus original product (A) were not similar. Finally , IV/IV correlation was not available for all products.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conficts of interest.

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References

[1] **Henning H. B.; Barbara, S. S**. The biopharmaceutics Richard, F., Michelle, A. C., and Luigi X. C., Pharmacology (Lippincott's Illustrated Review) , 4th edition , 2011 .

[2] **James M R., Lionel D L., Timothy GK M., and Albert F.,** A Textbook of Clinical Pharmacology and Therapeutics , 5th edition , 2008 .

[3] **Shopit A, Al-Adhal A, Sheiban AK, Amood M, Al-Kamarany**.(2014). Effect of calcium, alfacalcidol, andhemodialysis on secondary hyperparathyroidism. *Int J Pharm Pharm Sci* ;6 Suppl 2:267-72

[4] European Pharmacopoeia (EP). European Directorate for the Quality of Medicines. 6th Edition, 2008 .

[5] Unites State Pharmacopoeia (USP) . Unites States Pharmacopoeia Convention , 33, 2010.

[6] British Pharmacopoeia . British Pharmacopoeia Commission Secretariat of the Medicines and Healthcare products Regulatory Agency , 2009 .

[7] **Gindler M and King JD. (1972**). Rapid colorimetric determination ofcalcium in biologic fluids with methylthymol blue. *Am J ClinPath* . 58 (4) :376-382.

[8] **Caporal Gautier J., Nivet J. M, Algranti P., Guilloteau M, Histte M., Lallier M., N'guyen -Huu J. J., and Russotto R**.(1992).Guide de validation analytique. Rapport d'une commission SFSTP. II : Exemples d'application = Guide to analytical validation. Report of an SFSTP commission.II. Examples of application. *STP pharma pratiques*. 1992, 2 , 227 – 239 .

[9] **El Karbane, M., Azougagh, M., Amood A L-Kamarany, M., Bouchafra, H., Cherrah, Y., & Bouklouze, A.** (2014). Development and validation of a reversed-phase HPLC method for simultaneous analysis

of butylhydroxyanisol, simvastatin and its impurities in tablet dosage forms. *Annales pharmaceutiques francaises*, 72(4), 244–255.

[10] **Elkarbane, M., Al-Kamarany, M. A., Bouchafra, H., Azougagh, M., Cherrah, Y., Hubert, P., ... & Bouklouze**, A. (2015). Total error-based validation including the experimental design-based robustness evaluation of a stability-indicating method for the simultaneous quantification of hydrochlorothiazide and valsartan in tablet formulations. Acta *Chromatographica*, 27(2), 195-215.

[11] **Al-Kamarany MA, Alwosaby AS, El Karbane M, Karrouchi K** (2013) SFSTP guideline 1992- based validation of direct potentiometric method for quantification of sodium bicarbonate in baby's bliss gripe water available in Yemen. *Int J Pharm Pharm Sci 5*: 411-413.

[12] International Conference on Harmonization of Technical Requirement for the Registration of Pharmaceuticals for Human Use Topic Q2 (R1) : Validation of analytical procedures : Text and Methodology , Geneva , 2005 .

[13] **El Karbane, M., RamLi, Y., Al-Kamarany, M., Bouchfra, H., Radi, M., Karrouchi, K., Cherrah, Y., Bouklouze, A., & Azougagh, M.** (2014). Development and validation of HPLC dissolution assay of simvastatin tablets under normal and accelerated conditions. *J. Chem. Pharmaceu. Res.*, 6(6), 886-893

[14] **Al-Kamarany, M. A., El Karbane, M., Belomaria, M., Azougagh, M., Hubert, P. H., Alanazi, F., ... & Bouklouze, A.** (2012). Development and validation of LC method for the diclofenac sodium release determination using accuracy profile concept. *Asian Journal of Chemistry*, 24(10), 4393.

[15] **Al-Kamarany, M. A., Karbane, M. E., Ridouan, K., Alanazi, F. K., Hubert, P., Cherrah, Y., & Bouklouze, A.** (2012). Transfer of drug dissolution testing by statistical approaches: Case study. *Saudi Pharmaceutical Journal*, 20(1), 93-101.